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The effect of intrathecal gabapentin on neuropathic pain is independent of the integrity of the dorsolateral funiculus in rats

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ABSTRACT

Aim: This study evaluates the contribution of inhibitory pain pathways that descend to the spinal cord through the dorsolateral funiculus (DLF) on the effect of intrathecal gabapentin against spinal nerve ligation (SNL)-induced behavioral hypersensitivity to mechanical stimulation in rats.
Main method: Rats were submitted to a sham or complete ligation of the right L5 and L6 spinal nerves and a sham or complete DLF lesion. Next, the changes induced by intrathecal administration of gabapentin on the paw withdrawal threshold of rats to mechanical stimulation were evaluated electronically.
Key findings: Intrathecal gabapentin (200 μg/5 μl) that was injected 2 or 7 days after surgery fully inhibited the SNL-induced behavioral hypersensitivity to mechanical stimulation in sham DLF-lesioned rats; gabapentin was effective against the SNL-induced behavioral hypersensitivity to mechanical stimulation also in DLF-lesioned rats.
Significance: The effect of intrathecally administered gabapentin against SNL-induced behavioral hypersensitivity to mechanical stimulation in rats does not depend on the activation of nerve fibers that descend to the spinal cord via the DLF.

Introduction

The antiepileptic drug gabapentin ([1-(aminomethyl) cyclohexaneacetic acid]) has been used successfully in the treatment of patients with neuropathic pain (Backonja et al., 1998; Mao and Chen, 2000; Segal and Rordorf, 1996), and it is recommended as the first treatment option for the management of chronic pain conditions, such as diabetic neuropathy or post-herpetic pain (Attal et al., 2010; Dworkin et al., 2010; O'Connor and Dworkin, 2009). The clinical effectiveness of gabapentin has been confirmed in various animal models of thermal and mechanical hyperalgesia (Field et al., 1997; Luo et al., 2001; Pan et al., 1999; Partridge et al., 1998; Singh et al., 1996).

Although gabapentin is a gama-aminobutyric acid (GABA) derivative, it does not exhibit an affinity for GABA binding sites, including the GABA-A and GABA-B receptors (Taylor, 1997). Instead, it shows a specific binding affinity for the $\alpha_2\delta$ auxiliary subunit of voltage-dependent calcium channels (Brown and Gee, 1998; Field et al., 2006; Gee et al., 1996; Taylor, 2009; Wang et al., 1999). In fact, there is experimental evidence demonstrating that the antiallodynic effect of gabapentin is correlated with the up-regulation of $\alpha_2\delta$ subunits in the spinal cord and/or dorsal root ganglia (Luo et al., 2001, 2002).

The mechanism of the analgesic action of gabapentin is not fully understood. Several studies have focused on the analgesic action of gabapentin on the spinal cord (Abdi et al., 1998; Cheng et al., 2000; Cho et al., 2002; Chu et al., 2011; Hwang and Yaksh, 1997; Kaneko et al., 2000; Luo et al., 2001; Miletic and Miletic, 2000; Sandkühler, 2000; Sandkühler and Liu, 1998; Shimoyama et al., 1997). However, the effect of systemic gabapentin against neuropathic pain in mice was shown to be mediated by both supraspinal structures and the spinal cord (Tanabe et al., 2005). In addition, the supraspinal-mediated analgesic effects of gabapentin are correlated with activation of a descending pain inhibitory system that leads to the spinal release of noradrenaline (Takeuchi et al., 2007).

Intrathecal administration of gabapentin has been shown to be antinociceptive in rodents (Cheng et al., 2000; Kaneko et al., 2000; Takeuchi et al., 2007) and seems to act via a mechanism that is not mediated by the spinal release of noradrenaline and serotonin (Takeuchi et al., 2007). However, gabapentin injected intrathecally (Tanabe et al., 2005) or directly into the locus coeruleus (Hayashida et al., 2007) reduces neuropathic pain in rats; moreover, the effect is prevented by intrathecal administration of the α_2-adrenergic antagonist idazoxan.

The dorsolateral funiculus (DLF) is the main route through which inhibitory pain pathways descend to the spinal cord (Millan, 2002). Our present study evaluates the contribution of these specific

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inhibitory pain pathways on the anti-hyperalgesic effect of intrathecal gabapentin in a model of neuropathic pain in rats.

Materials and methods

Subjects

The experiments were conducted on male Wistar rats (140–160 g) from the main animal facility of the University of São Paulo (USP; Campus of Ribeirão Preto). The animals were housed two to a cage with free access to food and water. The rats were also maintained at a controlled temperature (23 ± 1 °C) with a 12-h light–dark cycle before and after surgery. The experiments were approved by the Commission of Ethics in Animal Research, Faculty of Medicine of Ribeirão Preto, University of São Paulo (Number 211/2005). The recommendations of the Committee for Research and Ethical Issues of International Association for the Study of Pain were followed throughout the experiments (Zimmermann, 1983).

Surgery

Spinal nerve ligation (SNL) and DLF lesioning were completed in sequence in rats anesthetized with tribromoethanol (250 mg/kg, i.p.). First, the right L5 and L6 spinal nerves were isolated and tightly ligated with a chromic catgut 5–0 suture as described previously (Kim and Chung, 1992). The incision was then closed with silk sutures. Next, the spinal cord was exposed by laminectomy at the T8 level, the dura mater was slit and the DLF lesion was made unilaterally by cutting a portion of the dorsolateral quadrant of the spinal cord ipsilateral to the SNL with a sharp knife while avoiding damage to the major blood vessels supplying the cord. Lastly, hemostasis was confirmed, the wound was packed with gelfoam and closed, and the animal was allowed to recover for 2 or 7 days before experiments. Sham-lesion rats were subjected to similar procedures except there was no ligation and/or DLF lesion. Rats that exhibited motor deficiency or a lack of increased sensitivity to innocuous mechanical stimulation were excluded from additional testing.

Intrathecal injection

Two or 7 days after surgery, each rat was anesthetized with isoflurane via a loose-fitting, cone-shaped mask, and catheterization of the spinal subarachnoid space was performed as previously described (Prado, 2003). Briefly, a 20-gauge Weiss needle was introduced through the skin into the L5-L6 intervertebral space. The correct positioning of the needle was assured by a typical flick of the tail or hind paw. A 12-mm length of polyethylene tubing (PE tubing, o.d. = 0.4 mm, dead space = 10 μl) was then introduced through the needle to protrude 2.0 cm into the subarachnoid space in a cranial direction. The needle was then carefully removed, the tubing was anchored to the back skin with a cotton thread suture, and anesthesia was discontinued.

Drug or saline was injected intrathecally in a volume of 5 μl over a period of 60 s followed by 5 μl of sterile saline at the same rate to flush the catheter. At the end of the experiment, the correct position of the catheter was determined by motor paralysis of the hind part of the animal occurring within 15 min after the intrathecal administration of 2% lidocaine (10 μl) followed by saline (10 μl). To further confirm the correct catheter positioning, 1% methylene blue (10 μl) was injected intrathecally, and the animal was then euthanized with an overdose of sodium thiopental and intracardial perfusion with saline followed by buffered formalin. Next, the T6–L6 spinal segment was removed, fixed in formalin, and cut through the L4–L5 plane. The DLF was identified using the atlas of Paxinos and Watson (2005), and the lesion was verified using 60-μm serial coronal sections stained with neutral red. Rats showing either the catheter tip positioned at sites other than the dorsal spinal cord or dye staining of the paravertebral musculature were not considered for data analysis. Gabapentin was purchased from Sigma (St. Louis, MO, USA).

Algesimetric testing

The mechanical threshold of rats was tested as previously reported (Prado and Dias, 2009). The rat was placed in an acrylic cage (12 × 10 × 17 cm) with wire-grid floors for approximately 15 min to allow behavioral acclimation. The threshold for mechanical stimulation was measured with an automated electronic von Frey apparatus (IITC Electronic Equipment, Woodland Hills, CA, USA) consisting of a hand-held probe unit connected to a rigid plastic tip (tip area 0.7 mm^2). The plastic tip was then applied with increasing force in an upward direction against the central area of each hind paw. The end point was characterized by the removal of the paw followed by clear flinching movements. After paw withdrawal, the movement of the probe was stopped, and the intensity of the pressure was automatically recorded. A single trial consisted of 3 applications of the tip, once every 5 s in each hind paw. The mean of three readings was taken as the mechanical threshold for a particular timing. The animals were tested before SNL (BL1), immediately before catheter implantation (BL2) and then 2 h after (BL3) the catheter implantation. The intrathecal injection was performed 5 min after the BL3 test, and the test was repeated 15 min later, and then at 30-min intervals for up to 105 min.

A preliminary experiment was conducted to examine the changes in the mechanical threshold immediately before and 2, 7, 14, and 21 days after SNL in sham ($n = 6$) or DLF-lesioned ($n = 6$) rats. The threshold for mechanical stimulation of each rat was evaluated always in the morning between 9:00 and 10:00 h at each day of experiment.

Data analysis

The mechanical threshold is reported as the mean ± SD. Comparisons between the control (sham) and test groups were made using a multivariate analysis of variance (MANOVA) with repeated measures to compare the groups over time. The factors analyzed included treatments, time and the treatment × time interaction. A one-way analysis of variance followed by a Bonferroni post hoc test was conducted in the case of a significant treatment × time interaction. The analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). The values of F (and the respective degrees of freedom indicated as subscripts) are shown in Results. The level of significance was set at $p < 0.05$.

Results

Our first experiment examined changes in the mechanical threshold following an SNL in sham or DLF-lesioned rats. The threshold for mechanical stimulation of each rat was evaluated prior to surgery and 2, 7, 14, and 21 days after surgery. The results revealed that the mechanical threshold of the experimental groups did not significantly differ before the surgical procedure (Fig. 1A). In addition, a sham SNL in sham (control) or DLF-lesioned rats did not produce a significant change in the behavioral response to tactile stimuli over the entire observation period. In contrast, SNL rats displayed a significant reduction in the mechanical threshold beginning on the second day after surgery. This behavioral hypersensitivity to mechanical stimulation remained unchanged in sham DLF-lesioned rats throughout the observation period. Behavioral hypersensitivity to mechanical stimulation also occurred in SNL/DLF-lesions rats at post-surgical days 2 and 7; however, the threshold of the SNL/DLF animals displayed a gradual and significant return to pre-surgical values at post-surgical days 14 and 21. The data in Fig. 1A were significantly different in

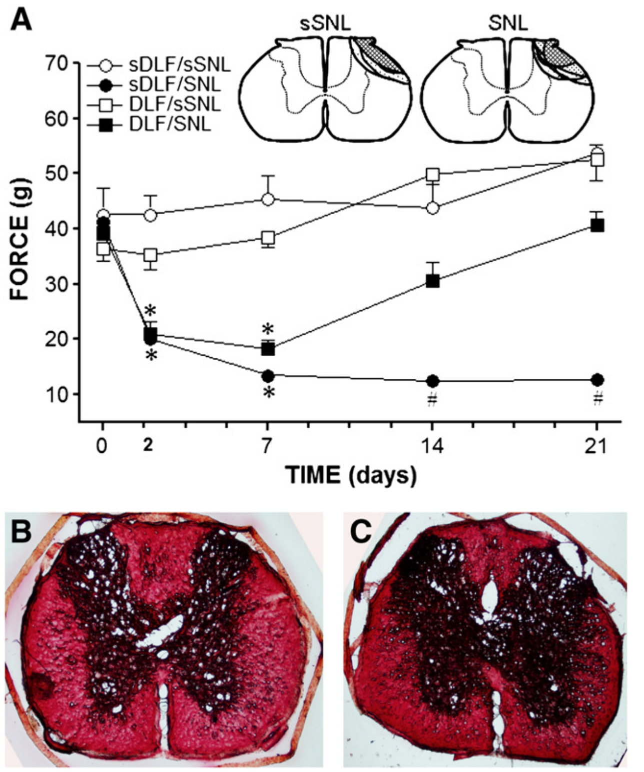

Fig. 1. Time course of changes in the mechanical threshold as measured in the plantar skin of the right hind paw of rats submitted to an ipsilateral sham (sSNL) or real (SNL) spinal nerve ligation and bilateral sham (sDLF) or real (DLF) lesion of the dorsolateral funiculus. Data in (A) are represented as the mean (± SD) of 6 rats per group. The extent of the DLF lesion is shown in the insert on images from Paxinos and Watson (2005). $p<0.05$ compared to sDLF/sSNL (*) or to the remaining groups (#) of animals. Representative panoramic microscope images taken from neutral red-stained spinal cord slices of sham DLF-lesioned and DLF-lesioned rats are shown in (B) and (C), respectively.

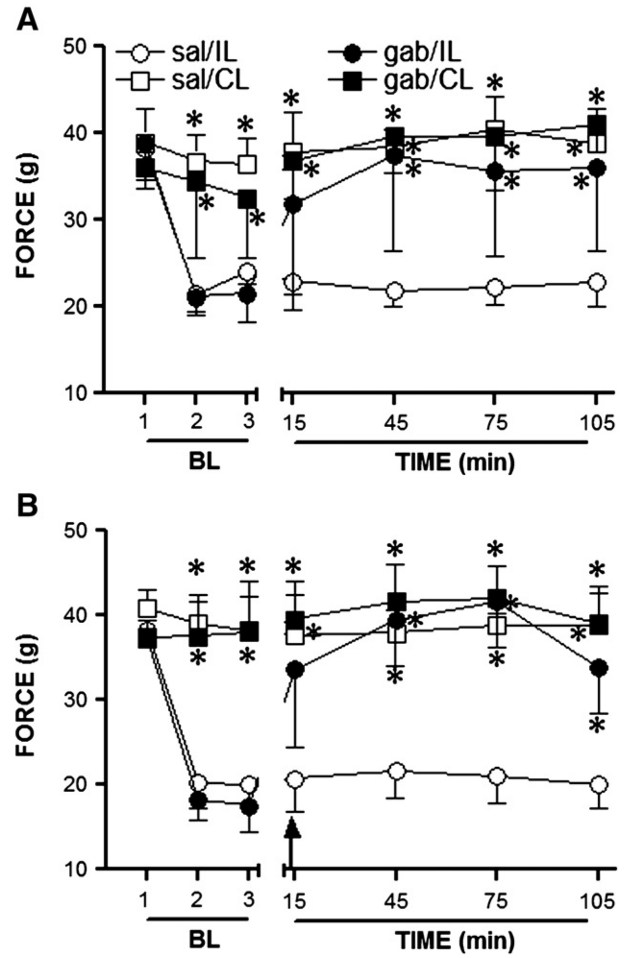

Fig. 2. Time course of the changes produced by the intrathecal injection of saline (sal = 5 µl) or gabapentin (gab = 200 µg/5 µl) on the mechanical threshold measured in the plantar skin of the right (IL) or left (CL) hind paw of SNL/sDLF lesion rats, 2 (A) or 7 (B) days after sham (square symbols) or real (circle symbols) ligation of the right L5 and L6 spinal nerves. The animals were tested before surgery (BL1), immediately before (BL2) and 2 h after (BL3) catheter implantation, and 15 min after intrathecal injection and then at 30 min intervals for up to 105 min. Data are represented as the mean ± SD of 5 animals. (*) Different from sal/IL group ($p<0.05$).

regards to treatment ($F_{7,40}=18.7$; $p<0.0001$) and time ($F_{4,160}=25.35$; $p<0.0001$). There was also a significant treatment × time interaction ($F_{28,160}=9.61$; $p<0.0001$). The extent of the DLF lesion (Fig. 1A, insert) shows that the tissue damage was restricted to the DLF and the adjacent dorsal horn. Representative panoramic microscope images showing sham DLF-lesioned and DLF-lesioned spinal cords are shown in Fig. 1B and C, respectively. The remaining experiments were conducted on rats 2 or 7 days after surgery because this time period displayed significant changes in the mechanical thresholds of SNL/DLF-lesioned and SNL/sham DLF-lesioned rats when compared with the control group.

Our next experiment examined changes induced by the intrathecal injection of gabapentin (200 µg/5 µl) or saline (5 µl) on the mechanical threshold of SNL/sham DLF-lesioned rats measured 2 (Fig. 2A) and 7 (Fig. 2B) days after surgery. Gabapentin- and saline-treated rats did not significantly differ in the threshold of their uninjured (contralateral) hind paw at any point during the study. Two days after surgery, a significant reduction of the mechanical threshold was observed in the injured (ipsilateral) hind paw of gabapentin- and saline-treated groups at BL2 and BL3 when compared with the threshold measured at BL1 or in the contralateral hind paw. This behavioral hypersensitivity to mechanical stimulation remained unchanged after the injection of saline, but it was completely attenuated by the injection of gabapentin (Fig. 2A). Similar results were obtained from experiments conducted 7 days after surgery (Fig. 2B). The curves in Fig. 2A and B were significantly different in regards to treatment ($F_{3,18}=51.8$ and 124.8, respectively; $p<0.0001$) and time ($F_{6,112}=9.0$ and 16.8, respectively; $p<0.0001$). There was also a significant treatment × time interaction ($F_{18,112}=88.0$ and 9.52, respectively; $p<0.0001$).

The injection of gabapentin (200 µg/5 µl) performed 2 days after surgery did not produce a significant change in the thresholds of ipsilateral or contralateral hind paws of sham SNL/sham DLF-lesioned rats (Fig. 3A) or sham SNL/DLF-lesioned (Fig. 3B) throughout the period of observation. The data displayed in Fig. 3A and B did not differ with respect to treatment ($F_{1,8}=0.0013$ and 0.15, respectively; $p\geq0.708$). There was no significant treatment × time interaction ($F_{6,48}=0.61$ and 0.15, respectively; $p\geq0.718$). The data shown in Fig. 3A, but not Fig. 3B, differ in regards to time ($F_{6,48}=2.27$; $p<0.021$ and $F_{6,48}=1.73$; $p=0.13$, respectively). The extent of the DLF lesion in sham SNL/DLF-lesioned rats was restricted to the DLF and the adjacent dorsal horn (Fig. 3C). Sham DLF lesion/SNL (Fig. 3D) or DLF lesion/SNL (Fig. 3E) rats showed no change in the baseline threshold of the contralateral paw; however, these rats had a significant reduction in the mechanical threshold of the ipsilateral paw. The behavioral hypersensitivity to mechanical stimulation induced by the SNL was fully reversed by an intrathecal injection of gabapentin (200 µg/5 µl) in both groups throughout the period of observation. The results shown in Fig. 3D and E differ with regards to treatment ($F_{1,8}=7.45$; $p=0.025$ and $F_{1,8}=16.27$; $p=0.0038$, respectively) and time ($F_{6,48}=4.5$; $p=0.0011$ and $F_{6,6}=10.61$; $p<0.0001$,

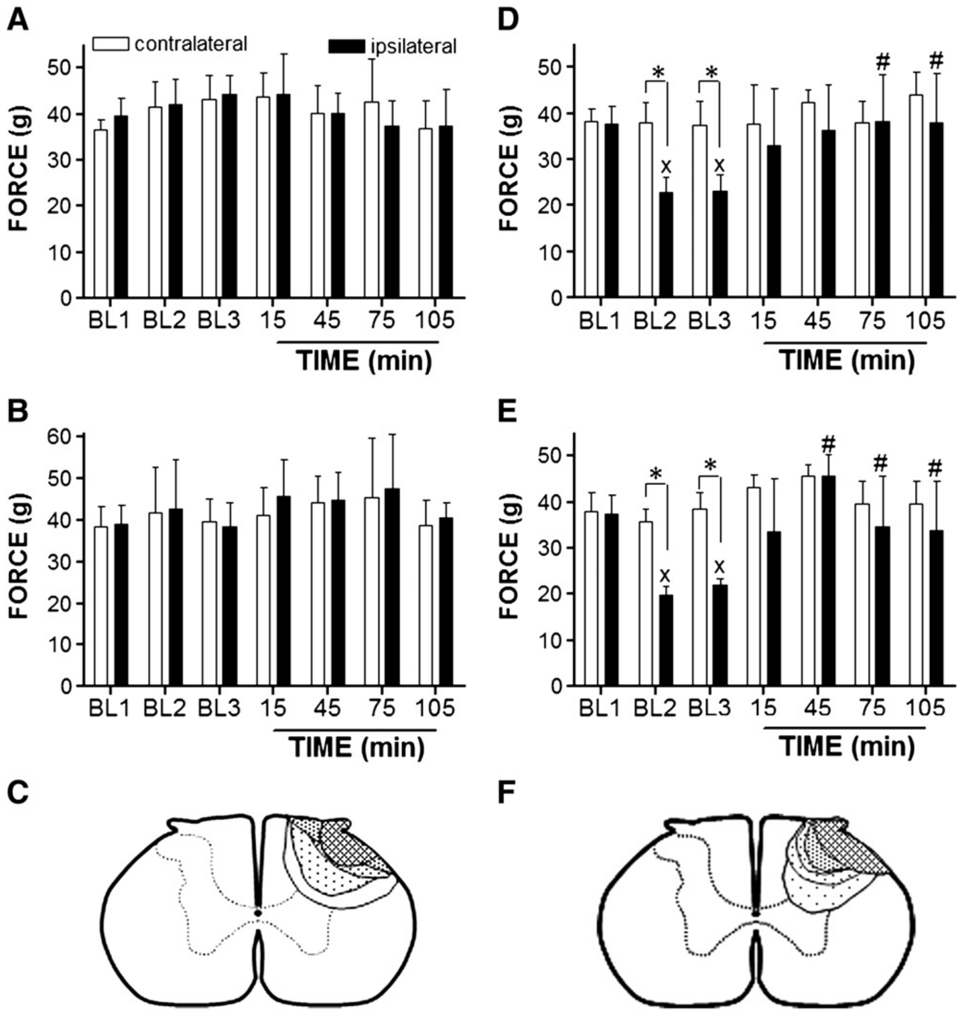

Fig. 3. Time course of the effects of an intrathecal injection of gabapentin (200 μg/5 μl) on the mechanical threshold measured in the plantar skin of the hind paws of rats 2 days after sham (A–C) or real (D and E) ligation of the right L5 and L6 spinal nerves. The experiments were conducted on rats with a sham (A and D) or real (B and E) DLF lesion. The animals were tested before surgery (BL1), immediately before (BL2) and 2 h after (BL3) catheter implantation, and 15 min after intrathecal injection and then at 30 min intervals for up to 105 min. The columns represent the mean ± SD of 5 animals per group. (*) different from the contralateral paw; (X) different from the contralateral and ipsilateral paws at BL1; (#) different from the ipsilateral paw at BL2 and BL3 ($p<0.05$). The extent of the DLF lesion in sham (C) and real spinal nerve ligated (F) rats is shown in images from Paxinos and Watson (2005).

respectively). The data displayed in Fig. 3E, but not Fig. 3D, were found to have a significant treatment × time interaction ($F_{6,48}=3.54$; $p=0.0055$ and $F_{6,48}=2.25$; $p=0.053$, respectively). The extent of the lesion in SNL/DLF-lesioned rats was restricted to the DLF and the adjacent dorsal horn (Fig. 3F).

Intrathecal gabapentin (200 μg/5 μl) that was administered 7 days after surgery did not produce significant changes in the mechanical threshold of the ipsilateral or contralateral hind paw of sham SNL/sham DLF-lesioned rats (Fig. 4A) or sham SNL/DLF-lesioned rats (Fig. 4B). The results displayed in Fig. 4A and B did not significantly differ in treatment ($F_{1,8}=1.42$ and 0.65, respectively; $p\geq 0.26$), nor had a significant treatment × time interaction ($F_{6,48}=0.19$ and 0.40, respectively; $p\geq 0.87$). The results shown in Fig. 4B, but not in Fig. 4A, differ regarding time ($F_{6,48}=11.72$; $p<0.0001$ and $F=1.66$; $p=0.15$, respectively). The extent of the spinal lesion in sham SNL/DLF-lesioned rats was restricted to the DLF and the adjacent dorsal horn (Fig. 4C). SNL/sham DLF-lesioned (Fig. 4D) or SNL/DLF-lesioned (Fig. 4E) rats showed a significant reduction of the mechanical threshold in the ipsilateral paw; however, no significant change was observed in the baseline threshold of the contralateral paw. The SNL-induced behavioral hypersensitivity to mechanical stimulation was fully reversed in sham- and DLF-lesioned rats with the administration of gabapentin throughout the period of observation. The data shown in Fig. 4D and F differ with regard to treatment ($F_{1,8}=31.64$ and 34.07, respectively; $p<0.0005$) and time ($F_{6,48}=14.74$ and 15.61, respectively; $p<0.0001$), and had a significant treatment × time interaction ($F_{6,48}=6.1$ and 9.05, respectively; $p<0.0001$). The extent of the lesion in SNL/DLF-lesioned rats was restricted to the DLF and the adjacent dorsal horn (Fig. 4F).

Discussion

Our results confirm that an SNL produces behavioral hypersensitivity to mechanical stimulation beginning 2 days after surgery that continues for at least 3 weeks as previously reported (Kim and Chung, 1992). In addition, a DLF lesion did not change the onset of the SNL-induced behavioral hypersensitivity to mechanical stimulation; however, this behavioral hypersensitivity to mechanical stimulation progressively returned to pre-surgical values at post-surgical days 14 and 21 as shown previously (Burgess et al., 2002). The experiments reported here were conducted 2–7 days after SNL, a period during which the mechanical thresholds of SNL/sham DLF- and SNL/DLF-lesioned rats were not significantly different. This was done because the onset of neuropathic pain is likely to be mediated by an increased afferent drive occurring shortly after the injury (Burgess et

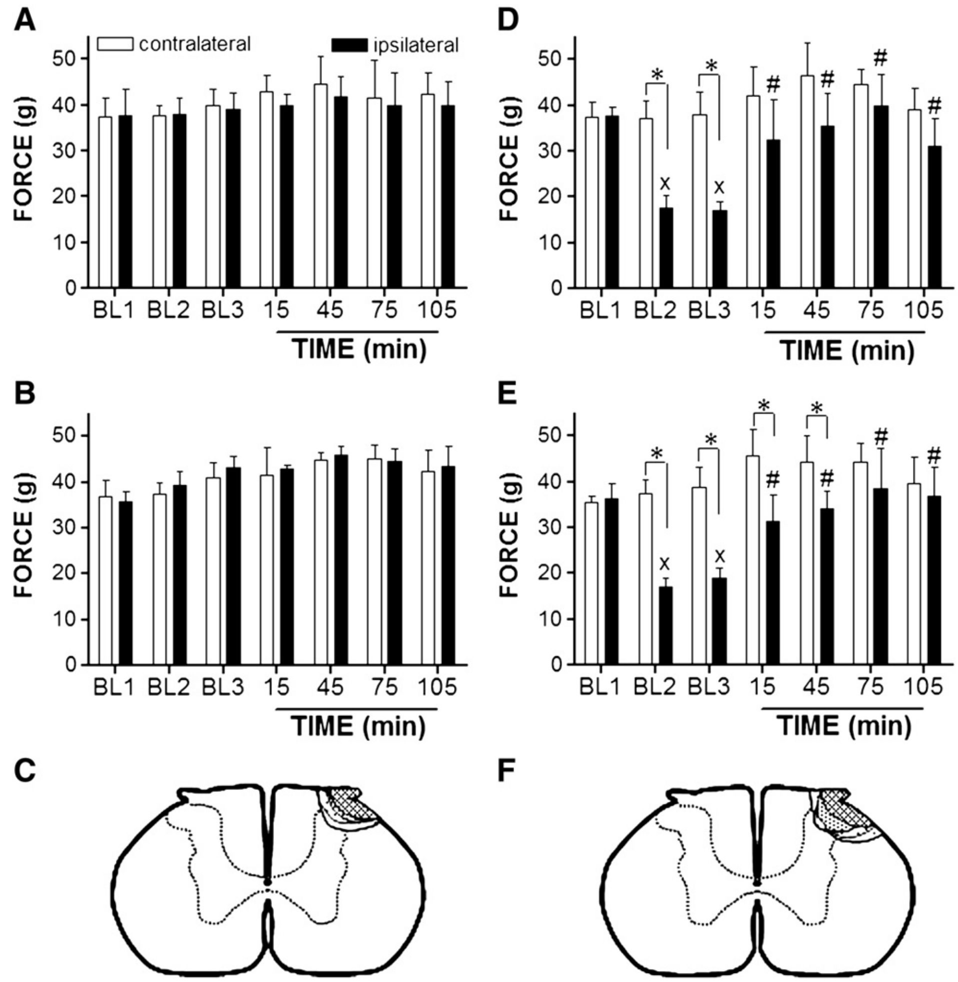

Fig. 4. Time course of the effects of intrathecal injection of gabapentin (200 μg/5 μl) on the mechanical threshold measured in the plantar skin of the hind paws of rats 7 days after a sham (A–C) or real (D and E) ligation of the right L5 and L6 spinal nerves. The experiments were conducted on rats with a sham (A and D) or real (B and E) DLF lesion. The animals were tested before surgery (BL1), immediately before (BL2) and 2 h after (BL3) catheter implantation, and 15 min after intrathecal injection and then at 30 min intervals for up to 105 min. The columns represent the mean ± SD of 5 animals per group. (*) different from the contralateral paw; (X) different from the contralateral and ipsilateral paws at BL1; (#) different from the ipsilateral paw at BL2 and BL3 (p<0.05). The extent of the DLF lesion in sham (C) and real SNL (F) rats is shown in images from Paxinos and Watson (2005).

al., 2002). The rostral ventromedial medulla (RVM) is a major source of descending axons that travel through the DLF (Millan, 2002), and it has been implicated in the inhibition and facilitation of spinal nociceptive transmission (Gebhart, 2004; Jones and Gebhart, 1988). The enhanced activity of afferent primary neurons is not enough to maintain the neuropathic state in the absence of a time-related development of descending facilitation arising from the RVM (Ren and Dubner, 2002).

Intraspinal gabapentin at doses above 300 μg causes no significant change in the motor function but produces significant sedation (Hwang and Yaksh (1997). By this reason, gabapentin 200 μg was chosen for the present study because it does not cause sedation. In addition, it did not change the mechanical threshold of sham SNL rats that had either a sham or real DLF lesion. In contrast, intrathecal gabapentin fully inhibited the SNL-induced behavioral hypersensitivity to mechanical stimulation in the injured hind paw of rats with a sham DLF lesion. This result was confirmed for injections of gabapentin performed at 2 or 7 days after surgery, and it corroborates earlier evidence that suggests that intrathecal gabapentin is efficient at mitigating neuropathic pain (Cho et al., 2002; Chu et al., 2011; Coderre et al., 2005; Hwang and Yaksh, 1997; Wallin et al., 2002). However, intrathecal gabapentin did not change the mechanical threshold of the uninjured hind paw nor the mechanical threshold of sham SNL/sham DLF-lesioned rats. The supraspinal administration of gabapentin has been shown to be analgesic only after peripheral nerve injury (Tanabe et al., 2005). We may then conclude that regardless of the site of action, the analgesic effect of gabapentin depends on the presence of persistent noxious input.

The effect of gabapentin against SNL-induced behavioral hypersensitivity to mechanical stimulation was not modified in animals with a previous DLF lesion. This result indicates that the integrity of the descending fibers projecting to the spinal cord via the DLF is not necessary for the spinal action of gabapentin against neuropathic pain. This conclusion diverges from the previous demonstration that gabapentin acts supraspinally to activate descending noradrenergic system in mice with peripheral nerve injury (Takasu et al., 2006; Takeuchi et al., 2007; Tanabe et al., 2005). Our experiment corroborates previous results that show that the spinally mediated analgesic effect of gabapentin is not accompanied by changes in spinal monoaminergic activities; therefore, the effect is not dependent on the evoked spinal release of noradrenaline and serotonin. However, the analgesia induced by intrathecal gabapentin is less intense after blockade of spinal α_2-adrenoceptors (Tanabe et al., 2005). It is likely that the effect of intrathecal gabapentin against neuropathic pain requires basal noradrenergic activity (Takeuchi et al., 2007).

Gabapentin binds specifically to the $\alpha_2\delta$-1 subunit of voltage-dependent calcium channels (Gee et al., 1996). Previously, dorsal root ganglion and spinal cord calcium channels were found to be up-regulated in a rat model of neuropathic pain (Li et al., 2004; Luo et al., 2001, 2002). Thus, an alternative mechanism for the anti-hyperalgesic effect of intrathecal gabapentin against SNL-induced behavioral hypersensitivity to mechanical stimulation includes a decrease in the spinal release of glutamate (Coderre et al., 2005), substance P and the calcitonin gene-related peptide (Fehrenbacher et al., 2003) by the terminals of primary afferent neurons via the blockade of calcium channels.

However, our present results do not exclude that the effect of spinal gabapentin SNL-induced behavioral hypersensitivity to mechanical stimulation is mediated by noradrenergic bulbospinal projections that descend to the spinal cord via the ventrolateral funiculus. In fact, noradrenergic innervation to the spinal cord is mainly provided by the locus coeruleus/subcoeruleus (Millan, 1999), and the stimulation-induced antinociception from the locus coeruleus is interrupted by lesioning of the ventrolateral funiculus (Tsuruoka et al., 2004).

Conclusions

The present study confirms that the spinal cord is a site of action for the mitigating effect of intrathecally administered gabapentin on SNL-induced behavioral hypersensitivity to mechanical stimulation in rats, and it excludes that the effect depends on the activation of nerve fibers that descend to the spinal cord via the DLF.

Conflict of interest statement

The authors declare no conflicts of interest.

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